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## REVIEW ARTICLE

# Is preimplantation genetic diagnosis the ideal embryo selection method in aneuploidy screening?



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**Abstract** To select cytogenetically normal embryos, preimplantation genetic diagnosis (PGD) aneuploidy screening (AS) is used in numerous centers around the world. Chromosomal abnormalities lead to developmental problems, implantation failure, and early abortion of embryos. The usefulness of PGD in identifying single-gene diseases, human leukocyte antigen typing, X-linked diseases, and specific genetic diseases is well-known. In this review, preimplantation embryo genetics, PGD research studies, and the European Society of Human Reproduction and Embryology PGD Consortium studies and reports are examined. In addition, criteria for embryo selection, technical aspects of PGD-AS, and potential noninvasive embryo selection methods are described. Indications for PGD and possible causes of discordant PGD results between the centers are discussed. The limitations of fluorescence *in situ* hybridization, and the advantages of the array comparative genomic hybridization are included in this review. Although PGD-AS for patients of advanced maternal age has been shown to improve *in vitro* fertilization outcomes in some studies, to our knowledge, there is not sufficient evidence to use advanced maternal age as the sole indication for PGD-AS. PGD-AS might be harmful and may not increase

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the success rates of *in vitro* fertilization. At the same time PGD, is not recommended for recurrent implantation failure and unexplained recurrent pregnancy loss.

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## Introduction

Despite the success of *in vitro* fertilization (IVF) as a treatment modality of infertility, several obstacles remain, including recurrent implantation failure and multiple pregnancies with their associated complications. Because both problems are directly related to the ideal goal of achieving implantation and ultimately the successful delivery of a single newborn, the selection and the appropriate number of embryos to be transferred are the objectives of the current studies.

Because the number and quality of the embryos to be transferred depends on the center's own strategy and/or the country's ethical and religious traditions, there are many different methods and approaches used throughout the world. Most methods only assess the developmental potential and morphological characteristics of the embryo, and do not provide sufficient information about genetic characteristics. Genetic studies of human gametes and embryos have shown that chromosomal abnormalities are the main problem in developmental arrest during the preimplantation period in embryos, implantation failures, and early pregnancy losses [1].

## IVF and embryo selection

A successful IVF procedure can be described as one that results in a singleton pregnancy with the subsequent delivery of a healthy newborn. In general, the success rates of IVF across the world are lower than expected, but they have been increasing in recent years with the use of newer technologies. A close examination of the data shows that many centers with high success rates also have high multiple pregnancy rates. Clinically, high multiple-pregnancy rates present new problems in patient management. Pregnancy rates are a good metric for assessing the center's general laboratory conditions and practices in terms of the developing embryo potential [2]. In addition to the obvious quality of gametes and associated embryo development, IVF failure and multiple pregnancies can be attributed to embryo selection and the number of transferred embryos. Currently, the most effective embryo selection methods are the morphological assessment of gametes and embryos, embryo transfer within a specific time interval, and a cumulative scoring system based on these factors [3,4].

## Noninvasive embryo selection methods

The most widely used embryo selection methods are based on pronucleus-stage evaluation, early cleavage assessments, the number and morphology of blastomeres in cleavage-stage embryos, potential evaluation of blastocyst formation, and the administration of one or more criteria in

selected cases [5]. Irrespective of the method used, embryo selection must provide useful information about the embryo development potential, and the duration of assessment must be expedient to protect the embryo from environmental factors. The laws and ethical values of the country and the laboratory conditions are equally significant in the selection of embryos.

## Evaluation of the quality of gametes

Most studies on assessment of the gamete quality in IVF practice aim to examine the link between the morphological features of oocytes and the success of the treatment. Previous studies indicate that oocyte morphology has no significance on fertilization, embryo quality, and implantation. There are other studies indicating that case-based and autonomous morphological abnormalities play an important role in pregnancy rates [6–10].

Studies carried out in the near term showed that the early cleavage period of embryo development is influenced by inherent oocyte factors of maternal origin. Therefore, oocyte quality is also directly related to the pronucleus-stage and early cleavage-stage embryo development. By comparison, effects influenced by sperm quality are seen after the four- to eight-cell stage when the embryonic genome is activated [11].

## Evaluation of pronucleus-stage embryos

Evaluation of pronucleus-stage embryos used the following criteria: (1) number, size, and symmetry of the pronuclei; (2) pronuclei settlement according to the polar bodies; (3) the number and distribution of pronuclear bodies; (4) the view of the nuclear membrane and the cytoplasm (the presence of cytoplasmic halo). It has been demonstrated that embryo development and implantation are significantly different when categorized into the different groups according to these criteria [12–14].

## Cleavage-stage embryo evaluation

Detection of early cleavage, division rate, the shape and size of blastomeres, the rate of fragmentation, number of nuclei in blastomeres, cytoplasmic image, features of perivitellin area (PVA), and zona pellucida are evaluated in the early cleavage stage.

## Evaluation of blastocyst-stage embryos

Another approach used for embryo selection is the selection of blastocyst-stage embryos. The purpose of this

approach is to increase pregnancy rate by growing embryos to the blastocyst stage *in vitro*, thereby eliminating cleavage-stage embryos without implantation potential. In addition, other parameters such as observed development pauses or stops are evaluated for embryo selection.

This approach gives very successful results in a particular set of patients. However, different groups reported that treatment might be cancelled in some cases due to the lack of good quality early cleavage-stage embryos [15]. In addition, blastocyst-stage embryo selection and transfer may not offer additional advantage for embryos with good implantation potential that subsequently resulted in early pregnancy loss. According to the recent Cochrane review, which evaluated nine randomized controlled studies, there is no significant difference between blastocyst transfer and early cleavage-stage embryo transfer in terms of abortion rates [16].

A study investigating the incidence of aneuploidy and mosaicism on Day 4 embryos showed that preimplantation embryos on Day 4 have many abnormalities, and self-correction does not occur at this stage of development. Perhaps self-correction may occur in the later stages of development. This study provided important information about the origin of aneuploidy in human embryos, however it was not ideal as only a limited number of embryos were investigated. Aneuploidy is often explained due to the recession and nondisjunction in anaphase. In some cases, aneuploidy can be caused by endoduplication due to the cellular division of a multipolar axis [17].

Therefore, aneuploidy screening (AS) is considered as an additional selection criterion in some centers to eliminate abnormal embryos and to increase healthy ongoing pregnancy rates in suspected cases.

## Preimplantation embryo genetics

Formation of human haploid gamete cells from diploid germ cells occurs during gametogenesis. Although all four haploid cells originate from diploid germ cells, transformation into functional gamete cells occurs during spermatogenesis. The cytoplasm is collected from within a single large gamete cell (the oocyte), and polar bodies do not have any biological function in terms of embryo formation and development. Haploid gamete cells from the male and female combine to form the diploid genome of the embryo during fertilization. After multiple mitotic divisions, the fertilized oocyte develops into a blastocyst on Day 5 or Day 6. Implantation occurs after hatching of the blastocyst through the zona pellucida [18]. Chromosomal abnormalities occur due to errors during meiosis and mitosis during gametogenesis and embryonic development. These errors represent the most significant impediments to achieving a healthy pregnancy. Studies show that the human gamete cells have aneuploidy rates of 12–37% for oocytes and 1–6% for sperm [19,20]. The most current hypothesis regarding the etiology of aneuploidy in oocytes is a “two-hit” hypothesis [21,22]. According to this hypothesis, the “first hit” can be defined as no recombination, or poorly formed recombination in the pair of homologous chromosomes during oocyte maturation. The oocytes cannot detect these recombination errors in their later stages of maturation, and the incorrect distribution of

chromosomes (the “second hit”) during meiosis I and II occurs. Studies indicate that many external (smoking, environmental toxins, etc.) and internal (advanced maternal age, reactive oxygen species formation, etc.) factors have an effect in the formation of this second stage. Errors that occur during meiosis often result in aneuploidy in all the blastomeres of the embryo. Mitosis errors in the early cleavage-stage embryos result in aneuploidy in some of the blastomeres, giving rise to blastomeres of the same embryo with different chromosomal constitution (mosaicism). Recent studies indicate that aneuploidy occurs in 50% of embryos from IVF methods [23,24].

## Preimplantation genetic diagnosis for AS

Preimplantation genetic diagnosis (PGD) is a technique used to determine the genetic defects in embryos created through IVF before their transfer to the uterus. Maternal age >35 years, patients with previous IVF treatment that resulted in trisomic conception, recurrent pregnancy loss, failed IVF treatments despite morphologically and high quality embryo transfer, HLA matched embryo selection for siblings, sex selection for specific diseases and cultural purposes are the main indication for PGD [25].

PGD was used to screen for X-linked diseases, and the first successful case was reported by Handyside et al. [26] in London in 1989. The authors reported PGD as a promising technique for the detection of X-linked diseases, and subsequently, it has been used for single-gene disorders, translocations, and Mendelian disorders.

In IVF laboratories, embryo development is usually monitored on Day 3 (cleavage stage) to Day 5 or Day 6 (blastocyst stage). Chromosomal abnormalities have been detected in >50% of the cleavage-stage embryos. These abnormalities reach 80% in female patients aged 42 years and older [27,28]. Some of the abnormal embryos have developmental arrest between Day 3 and Day 5 [29]. However, the majority of the abnormal embryos continue to develop. Even in patients of advanced maternal age, 40% of abnormal embryos reach the blastocyst stage [30].

The goal of PGD-AS is the selection of cytogenetically normal embryos, which in turn, results in embryos with a higher chance of survival. Ultimately, this will increase the likelihood of implantation and the number of term pregnancies [31].

Following the study by Handyside et al. [26], PGD-AS has been used for patients of advanced maternal ages undergoing IVF treatment [32]. Aneuploidy results in lower implantation rates and higher abortion rates. It causes recurrent implantation failure and recurrent abortions even if the transferred embryos have normal morphology. Therefore, PGD-AS is expected to reduce the rate of recurrent abortion, and increase the implantation rates. Although some studies reported improved pregnancy outcomes following PGD [32–36], other studies did not demonstrate an improvement in implantation and live-birth rates [37–40]. Verlinsky et al. [40] showed that PGD is an important technique for the screening of embryos for genetic and chromosomal disorders such as unbalanced translocations, Mendelian genetic diseases, and HLA typing. Although they showed increased implantation and

decreased abortion rates, no improvement on the live-birth rate was reported.

Carp et al. [41] indicated that PGD may be more effective in older age groups because abnormal embryonic karyotypes are seen more commonly in this group of patients. Recurrent pregnancy loss has two different subgroups, arising from normal or abnormal embryos. PGD can improve pregnancy outcomes in patients who have recurrent pregnancy loss arising from abnormal embryos. However, PGD is not useful in unexplained recurrent pregnancy loss with the exception of the embryos without translocations. In the embryos without translocations, the subsequent pregnancy is likely to have a good prognosis in patients with recurrent pregnancy loss arising from abnormal embryos [38]. Pregnancy rates of approximately 70% have been reported in patients who had three recurrent miscarriages, and 60% in patients who had four recurrent miscarriages [42].

Harton et al. [43] evaluated the relationship between maternal age, chromosomal abnormality, implantation, and pregnancy loss. In their study, selective transfer of euploid embryos showed that implantation and pregnancy rates were not significantly different between reproductively younger and older patients up to the age of 42 years. Some patients do not have euploid embryos available for transfer, a situation that increases with advancing maternal age. There are a lot of data suggest that the dramatic decrease in IVF treatment success rates with female age is primarily caused by aneuploidy.

PGD-AS is not limited to PGD, and this issue had been an understudy before the PGD methods become commonplace. Initial experiments to screen for aneuploidy were first reported in polar bodies in 1995, and in the cleavage-stage embryos in 1997 [44–46]. It has been reported that an increasing number of centers are using newer techniques every year, and there are many studies on the subject from its initial application to the present. However, despite the increasing use of PGD-AS, a recently conducted meta-analysis questioned the effectiveness of the PGD-AS method [47]. The meta-analysis and other recently published studies indicate that the underlying reasons for the success or failure of PGD-AS are thought to be the result of different variables such as suboptimal embryo culture conditions, biopsy techniques, the number of cells received, fixation method, examined chromosome numbers, the center's technical expertise, and PGD (variable due to the formation of sperm and oocytes, age-associated increase in aneuploidy, and mosaicism).

### European Society of Human Reproduction and Embryology PGD Consortium: 10-year data summary

The European Society of Human Reproduction and Embryology PGD (ESHRE PGD) Consortium was established in 1997. The goals of the consortium are the safe use of PGD, embryo safety, ethical issues related to human embryos, and providing PGD data on a regular basis. The ESHRE PGD Consortium gathers data from its member centers and genetic diagnosis laboratories to analyze and share. As such, it provides PGD guidelines for health-care professionals.

This consortium has investigated 27,630 PGD cycles from January 1997 to 2007. PGD techniques were utilized as follows: 61% for AS, 17% for single-gene disorders, 16% for chromosomal abnormalities, 4% for X-linked diseases, and 2% for sex selection due to cultural reasons. Only 10% of cycles were for chromosomal disorders, and cultural sex selection data were not reported [48].

According to the reported data, 4253 cycles of inherited chromosomal abnormalities were evaluated during the oocyte retrieval stage. Reciprocal translocations were more common than Robertsonian translocations in terms of PGD indications. Robertsonian translocation carriers had higher implantation and pregnancy rates when compared with reciprocal translocation carriers.

Cystic fibrosis, diabetes mellitus type 1, myotonic dystrophy type 1, Huntington disease, beta thalassemia, sickle cell anemia, fragile X syndrome, spinal muscular atrophy, Duchenne muscular dystrophy, neurofibromatosis type 1, hemophilia, familial adenomatous polyposis, Charcot–Marie–Tooth disease, familial amyloidotic polyneuropathy, Marfan syndrome, tuberous sclerosis, and von Hippel–Lindau disease were the most frequent indications for PGD according to the Consortium's report [48].

Both failures of PGD practices and the successful application of PGD practices have been reported previously. Most experts agree that the main factor for the failure of PGD is the existence of chromosomal mosaicism in early screening embryos, where embryo biopsy is not representative of the other blastomeres.

Currently, array comparative genomic hybridization (A-CGH) and single-nucleotide polymorphism (SNP) microarrays are the commonly used genetic diagnostic methods for PGD applications. The cost of each method, experience of the laboratory personnel, the standardization of the tests, as well as the effectiveness and reliability of the tests are important considerations when making a decision on using these methods, as well as for the subsequent interpretation of these data.

### Fluorescence *in situ* hybridization analysis of embryos

Analyzing one or two of the blastomeres from Day 3 embryos, cytotrophoblasts from the blastocyst-stage embryos, and polar bodies from the oocytes with five to 10 fluorescence *in situ* hybridization (FISH) probes provides useful information for PGD [49–51]. The FISH method with the use of probes for the evaluation of chromosomes is the most frequently used diagnostic tool for cytogenetic analysis of embryos. The FISH method allows chromosomal analysis in the interphase stage, without the need for cell culture and metaphase spread that are required in conventional cytogenetic studies. FISH allows for simultaneous analysis of five to nine chromosomes using a consecutive hybridization process [52,53]. One or two cells are taken from embryos to be investigated. The cell(s) are lysed and fixed onto a glass slide to allow the DNA probes to reach the nucleus. It has been suggested that the success of PGD-AS applications can be increased by further examining additional chromosomes [54].

Baart et al. [55] carried out a study in 2007 in which they conducted three sequential FISH analysis of 15 chromosomes



in 52 pairs of embryos that were donated for research purposes by couples who achieved pregnancy after IVF treatment. It was observed that the examination of seven more chromosomes, in addition to the nine routinely examined, did not change the outcome in terms of aneuploidy rates. However, the mosaic embryo percentage increased. The results raise the necessity of further investigating the implantation potential of the mosaic embryos, and the effect of mosaic structure on pregnancy rates.

PGD result differences between centers are likely to be affected by variability in biopsy techniques and genetic testing [56]. For example, some researchers found that two-cell biopsy from the cleavage-stage embryo showed no improvement in the outcome of IVF. They showed that two-cell biopsy is harmful to the embryos, and that there are less harmful effects when single-cell biopsy is used [57,58].

## Evaluating the effectiveness of PGD for aneuploidy

Mastenbroek et al. [59] compared cases using PGD and non-PGD in terms of ongoing pregnancy rates [37–69%, 95% confidence interval (CI): 0.51–0.93] and live-birth rates (35–24%, 95% CI: 0.50–0.92), respectively. They propose that Day 3 biopsy procedure decreases implantation rates compared with the control group, even if the biopsy procedure was performed adequately [60,61]. They further propose that blastocyst biopsy is less detrimental than Day 3 biopsy.

It is well-known that maternal age is closely related to the rate of aneuploidy in oocytes, especially in women over the age of 40 years where aneuploidy rates reach up to 60%. In the aforementioned study, the authors observed that oocytes from young fertile women have a low aneuploidy rate (3%).

Rubio et al. [62] investigated the effectiveness of PGD by FISH analysis for two different indications, namely, advanced maternal age and recurrent pregnancy loss. In this prospective study, patients were divided into two groups: Day 5 blastocysts with PGD, and Day 5 blastocysts without PGD. Day 3 biopsy was used for AS. In the PGD group, the live-birth rates increased 2.5-fold compared with those without PGD (95% CI: 1.26–5.29). However, there was no statistically significant difference in terms of recurrent pregnancy loss between the two groups. PGD with FISH method has been found to be useful for advanced maternal age. In contrast to the results of this study, Debrock et al. [63] found no difference between PGD and the control group in advanced maternal age patients (>35 years) in terms of clinical implantation rate (15.1%, 14.9%, rate ratio 1.01; exact CI: 0.25–5.27), ongoing pregnancy rate up to 12 weeks (9.4%, 14.9%), and live-birth rate per embryo transfer (9.4%, 14.9%, rate ratio, 0.63; exact CI: 0.08–3.37). They concluded that the hypothesis of “PGD improves pregnancy outcomes for advanced maternal age” was not confirmed. According to the American College of Obstetricians and Gynecologists’ report published in 2009, there is not enough evidence to propose PGD solely for women of advanced maternal age and for those undergoing AS. According to the report of the committee, PGD-AS does

not increase the success rates of IVF, and it may be harmful. In addition, PGD is not recommended for unexplained recurrent pregnancy loss and recurrent implantation failure [64].

Another study investigated whether uniparental disomy could represent an outcome of embryonic aneuploidy self-correction and its relevance to PGD. They found that uniparental disomy is extremely rare and routine screening during PGD may not be necessary [65].

## Misdiagnosis after FISH testing

According to the ESHRE PGD Consortium data, the FISH method was used for PGD in 21,829 cycles. A total of 15,981 embryo transfer procedures were performed for chromosomal abnormalities, pregenetic diagnosis, X-linked diseases, and social gene selection using the FISH method. A total of 16 misdiagnoses have been reported, which accounts for 0.1% of the transferred embryos. The error rate of chromosomal rearrangement was 0.1% (only 3 cases were diagnosed incorrectly in 2731 cases) [48].

There are many causes of misdiagnosis that are specific to single-cell preimplantation FISH testing. Limitations exist in both the technology and biological factors related to the embryos. Technical limitations are overlapping FISH signals, hybridization failure, nonspecific hybridization, and the difficulty in interpreting closely adjacent signals. The inherent complexities of the biology of the embryo also contribute to misdiagnosis after FISH. It is well-known that preimplantation embryos can be chromosomal mosaics, and that different cells may have a different chromosomal constitution. This could lead to adverse misdiagnosis when some cells are aneuploid and others are euploid.

Wilton et al. [66] reported that the causes of misdiagnosis are confusion of embryo and cell number, transfer of the wrong embryo, maternal or paternal contamination, allele dropout, use of incorrect and inappropriate probes or primers, probe or primer failure, and chromosomal mosaicism. Unprotected sex has been mentioned as a cause of adverse outcomes not related to technical and human errors. They indicated that a majority of these causes can be prevented by robust diagnostic methods within laboratories working to appropriate quality standards. However, diagnosis from a single cell remains a technically challenging procedure, and the risk of misdiagnosis cannot be eliminated.

PGD testing for chromosomal rearrangements using FISH is affected by additional difficulties. An individualized panel of FISH probes must be devised to detect all possible segregants of the translocation. At least one misdiagnosis reported to the Consortium occurred when the FISH protocol was unable to detect some unbalanced forms of the translocation [66].

The FISH technique is mostly used for the determination of aneuploidy and translocations. It may lead to different results because of a limited number of chromosome examinations by the FISH method, different interpretation of results, and improper use of biopsy and fixation techniques. Routine FISH analysis examines only one third of the chromosomes, and there may be an aneuploidy on the other unprobed chromosomes. Therefore, the FISH

technique for PGD has limitations for the detection of chromosomal abnormalities, because the normal results of a small number of chromosomes do not rule out abnormalities on other nontested chromosomes [67]. The A-CGH method, which allows for examination of all the chromosomes, is therefore a more effective method than the FISH technique [68,69].

## A-CGH

As an alternative to FISH, the A-CGH method makes it possible to analyze all chromosomes in embryos. DNA microarray technologies measure hybridization between the patient's DNA (the "target") and a matrix of known DNA sequences (the array "features") immobilized on a solid-state matrix. Depending on the array platform and hybridization protocol, microarray can reveal gains or losses of genome segments, or determine the patient's genotype for SNPs. In cases of aneuploidies, balanced translocations, and complex karyotypic disorders with multiple rearrangement, A-CGH is more effective than FISH [70–72]. A-CGH has been used in many PGD centers around the world and data are available that will ultimately improve the outcome of PGD. The ESHRE PGD group continues to work on identifying best practices for A-CGH and polar body biopsy [48,73]. In this procedure, target and control genomic DNAs are mixed and competitively hybridized to the same array. Changes in the hybridization ratio of target to control at a region indicate a gain or loss of material relative to the control genome. Because A-CGH examines every chromosome, and reveals events below the limits of microscopic detection, it is able to identify chromosome anomalies that a standard eight- or 12-chromosome FISH might fail to detect. However, A-CGH does not detect balanced rearrangements or triploidy, where the target to control ratio of DNA hybridized to the array features is constant along the genome [70,71].

A-CGH allows visualization of 46 interphase chromosomes. The test sample, which is amplified with Whole Genome Amplification kit (WGA), is marked with a color. A normal reference sample is amplified in the same way, and it is marked with a different color. Probes used to hybridize metaphase-stage chromosomes in the form of plaques, therefore A-CGH enables not only enumeration of all chromosomes but gives a more complete picture of the entire length of each chromosome and has demonstrated that chromosomal breakages and partial aneuploidies exist in embryos [74]. This allows simultaneous evaluation of all chromosome pairs [70,75]. Another advantage of the A-CGH over FISH is that unlike the FISH technique, preclinical validation is not required in A-CGH [70]. Fiorentino et al. [70] published 28 cycles, which evaluated cleavage-stage embryos by PGD for chromosomal translocation. Most of the embryos were diagnosed successfully (93%); 60% of the embryos were eligible for transfer per cycle. A 70% pregnancy rate was achieved per transfer. A-CGH merits further validation in the blastocyst biopsy. Single-gene disorders are amenable to whole-genome analysis using the SNP microarray. The SNP array features include alternative alleles for a large number of polymorphisms, and hybridization indicates which SNP allele(s) are present in the target genome. The SNP genotypes of two parents and a reference

child define maternal and paternal haplotype of a gene of interest, and linkage then establishes the genetic risk for a second child based on its combination of parental haplotypes [76]. Tan et al. [77] evaluated whether SNP array in combination with trophectoderm biopsy and frozen embryo transfer improves clinical pregnancy rates and compared the obtained results with traditional PGD based on FISH-PGD using blastomere biopsy and fresh embryo transfer for translocation carriers. A total of 169 couples underwent SNP analysis, including 52 Robertsonian translocation carriers and 117 carriers of reciprocal translocations. Reliable SNP-PGD results were obtained for 92.8% of biopsied blastocysts. The procedure using the SNP array combined with trophectoderm biopsy and frozen embryo transfer significantly improves the clinical pregnancy rate when compared with traditional PGD based on FISH (FISH-PGD) using blastomere biopsy and fresh embryo transfer for translocation carriers. The miscarriage rate also slightly decreases in the SNP array group. In conclusion, SNP arrays can detect both chromosome segmental imbalances and aneuploidy, and might overcome the limitations of FISH in PGD for translocation carriers.

## Conclusion

Aneuploidy in human embryos originates from errors in both mitosis and meiosis. The main purpose of PGD-AS application is achieving a healthy pregnancy as a result of screening embryos for chromosomal disorders, and selecting healthy embryos for transfer into the uterus. Cytogenetic examination of more chromosomes increases the chance of identification of abnormal embryos. By contrast, studies show that examination of more chromosomes increases the chance of identification of mosaic embryos. Because the development and implantation potential of mosaic embryos have not yet been elucidated, the effect of AS methods and their clinical significance require further studies in the future. Even if PGD-AS for advanced maternal age seems to improve IVF outcome in several studies, there is not sufficient evidence to support advanced maternal age as the sole indicator for PGD-AS. PGD-AS might be harmful and may not increase the success rates of IVF. In addition, PGD-AS for recurrent implantation failure, and unexplained recurrent pregnancy loss is not recommended.

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